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BUD SEPARATION IN *HYDRA OLIGACTIS*

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Abstract

Bud detachment with peduncle and basal disc formation distinguishes the asexual reproductive products of *Hydra* from the secondary axes of colonial coelenterates. This bud individuation was examined in *Hydra oligactis* using three approaches. Isolation experiments showed that parental body column could enhance hypostomal tissue's development of peduncle and basal disc. This established that the parental body column has a role in bud detachment. Grafting procedures used distal budding to indicate ability to direct bud separation. In bud formation and separation from the most distal surface of a subhypostomally amputated parental animal, the budding region of the parental body column was both necessary and sufficient for individuation. Cell quantification studies revealed a combination of three cellular characteristics in regions directing distal budding: a small proportion of nerve cells, a "big" interstitial-cell/epithelial cell ratio considerably <1, and an interstitial-cell derivative/epithelial cell ratio of <0.50.

Introduction

The freshwater coelenterate *Hydra* reproduces asexually by budding, forming replicate individuals. Among the significant questions raised by budding are the bases for the distinction between a new axis of symmetry that detaches (a hydra bud) and one that does not (leading to the formation of a colonial animal) (Webster, 1971). Hydra budding is usually divided into three distinct stages: initiation, elongation, and separation or individuation. Clarkson and Wolpert (1967) categorized the morphological changes that occur during this process, from a deformation of the parental animal's body column (the bud protrusion) to the elaboration of the mature bud before its detachment.

Grafting experiments between symbiotic and aposymbiotic *Hydra viridis* specimens established that the cell source for buds is at least partly parental. Epithelial cells of the epidermal and gastrodermal layers move down the parent body column and out into the bud (Shostak and Kankel, 1967). Non-epithelial cell types, such as nematocytes and interstitial cells (I-cells), most of which normally migrate distally, move quickly into any developing bud present (Herlands and Bode, 1974). The cellular composition of the developing bud also changes from an early homogeneous distribution of epithelial, interstitial, and nematoblast cells to the pattern of regional specialization characteristic of a nonbudding parent animal (Sanyal, 1967; Bode *et al.*, 1973).

Bud initiation and elongation have been investigated extensively. Bud initation appears to be related to a requisite quantity of parental cells in the budding region (Shostak *et al.*, 1968; Webster and Hamilton, 1972). Inhibiting mitosis and DNA synthesis by radiation does not interrupt the process, suggesting that bud elongation may be a tissue movement phenomenon (Clarkson and Wolpert, 1967). Campbell (1974) described some of the mechanics of this elongation process.

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Bud separation, or individuation, has long been recognized as a distinct event. Rand (1899) recognized a difference between lateral graft separations from a host animal and bud separation. His lateral grafts would eventually grow apart but they lacked the sequence of peduncle and basal disc formation seen in buds. Rand remarked that the conditions affecting bud constriction were a matter of conjecture. Seventy years later Webster (1971) noted the precise control of bud size at detachment, but also was unable to identify the cause of bud separation.

This investigation attempted to identify bud separation properties in *Hydra oligactis* by localizing the source of separation capacity and by describing conditions at that source when it directs bud detachment. Bud separation is accomplished by the formation of peduncle and basal disc; therefore, capacity to develop these structures was analyzed in various situations.

We also investigated the ability of tissues such as the bud hypostomal tissue, parent hypostomal tissue, and/or parental body column to direct distal bud detachment. The influence of these tissues on bud detachment was assessed by challenging them to direct bud separation from the distal region of a host animal, where budding does not normally occur (Webster, 1966; Tardent, 1972; Brooks, 1976).

Materials and Methods

Culture methods

H. oligactis were mass cultured in 8" fingerbowls according to the methods of Lenhoff and Brown (1970). The balanced saline solution (M solution) was prepared with distilled water and contained kanamycin sulfate to retard bacterial growth (Lesh-Laurie *et al.*, 1976). Animals were maintained in constant light and temperature (19 ± 1°C) and were fed to repletion each Monday, Wednesday, and Friday with *Artemia salina* nauplii. They were allowed to feed 4–6 h, after which they were cleaned of adherent debris and placed in fresh culture solution.

Experimental procedures

For experimental procedures, *H. oligactis* were taken from mass culture 1 day post-feeding. Several experiments involved excising tissue and then monitoring the isolated piece for peduncle and basal disc formation. Tissue pieces isolated included: a zero-to-two-tentacled bud with parental body annulus, a bud protrusion with parental body column, a zero-tentacled bud with parental body column, a four-tentacled bud with parental body column, parent and bud hypostomes and tentacle rings, and parent and bud axes isolated from a single animal. All tissue isolates were cultured at a density of 0.5 ml culture solution/isolate. Attachment to the container was considered evidence of a basal disc. Where parental body column was isolated with a developing bud, the development of the parental tissue also was noted. Isolate development was followed for up to 16 days.

In grafting, tissue surgically removed from a donor animal was transferred to a host animal. The donor and host tissue then were allowed to heal together. Two petri dishes were used, one of them modified to hold host and donor tissues in apposition during healing. Amputations were performed in one petri dish, which contained culture solution. The prepared host animal was transferred to the second dish, which had been layered with paraffin wax with human hairs embedded vertically in it, and which contained culture solution covering the projecting hairs.

To graft donor tissue to a host distal surface, nonbudding animals, 1 day post-feeding, were selected as hosts and amputated subhypostomally with a single clean

cut immediately proximal to the tentacle whorl. The remaining proximal body was transferred to the paraffinized dish. Using watchmaker's #5 forceps, the host was positioned so that a hair was aligned through the axis of the body column.

The desired donor tissue was then excised and transferred to the paraffinized dish. By using forceps that passed through the piece of donor tissue, it was placed in direct apposition to host tissue. Care was taken to place cut surfaces in contact and to align the axes of host and donor. Grafts were held together by an alcohol-cleaned loop of human hair and allowed to heal for 2–4 h. The hair loop then was removed and the graft gently lifted from its position on the hair.

To graft donor tissue to the lateral surface or to the bud of a host animal, the host animal was placed onto the hair so that the hair passed through the parent and/or bud, with the parent lying horizontally on the paraffin surface.

After they were removed from the paraffinized dish, the grafted samples were placed in a 1″ covered culture dish containing 5–7 ml of culture solution. Grafting success ranged from 50%, in grafts involving bud protrusions, to 100%, in grafts employing four-tentacled bud hypostomes or parental hypostomes as donor tissue. Grafted animals were monitored for budding activity for up to 14 days.

Cell counting

Cells were separated and counted using the maceration technique of David (1973). Ten tissue samples of each selected body region were macerated together and spread on a 3 × 1″ glass microscope slide. Cell counts were made by random edge-to-edge sweeps across the short dimension of the slide, thus eliminating any effects of selective cell accumulations at the slide edges.

Results

Peduncle and basal disc formation from isolated bud and parent tissue

Various portions of the parent and bud axes were isolated and monitored to identify the body regions of parents and buds that can form peduncle and basal disc. Table I indicates that: (1) bud tissue forms peduncle and basal disc more readily than does parental tissue (experiments 1–5); and (2) both parental and bud tissue form these structures more readily as the position of amputation moves

Table I

Isolation experiments.

Isolate type	Sample size	Number showing peduncle and basal disc formation
1. Parental hypostome and tentacle ring	41	0
2. Four-tentacled bud hypostome and tentacle ring	35	5
3. Two-tentacled bud hypostome and tentacle ring	32	4
4. Parental body column	55	27
5. Bud body column	56	51
6. Zero-to-two-tentacled bud with parental annulus	34	34
7. Bud protrusion with parental annulus	31	31
8. Four-tentacled bud with parental body	31	28
9. Zero-tentacled bud with parental body	35	33
10. Bud protrusion with parental body	44	43

proximally along the body column (experiments 4 and 5). Isolates that included a portion of parental tissue with the bud formed peduncles and basal discs most frequently (experiments 6–10). Thus, parental body column tissue may enhance the ability of a developing bud to form peduncle and basal disc.

Identification of the region which directs bud detachment

A series of grafting experiments examined bud separation more critically. These experiments were designed to identify the tissue directing detachment of a bud, and where along the distoproximal axis of an organism this tissue could exert its influence. We examined principally the ability of tissue to direct "distal budding," or bud formation and detachment from the most distal surface of a parental animal amputated subhypostomally. Distal budding rigorously tests the ability of grafted tissue to direct bud detachment.

Initially, presumptive bud hypostomes and bud hypostomes of various developmental stages were grafted to parental hosts amputated subhypostomally. Definitive bud hypostome tissue did not lead to distal budding: No distal budding was seen in 141 grafts when donor tissue was a four-tentacled bud hypostome, two-tentacled bud hypostome, or zero-tentacled bud hypostome. However, when donor tissue was a bud protrusion with an attached wedge of parental body column, 30 of 56 grafts produced distal budding. In the latter series of grafts, some parental tissue was included due to the small size of the presumptive bud at this early stage. These results suggest a requirement for parental tissue in directing bud detachment. However, bud tissue free from hypostomes also might have been responsible for the effect.

To test whether a large amount of bud tissue, free of bud hypostome, could direct distal budding, a two-tentacled bud axis was used as donor tissue. In none of 33 grafts did a bud axis alone lead to distal budding. A two-tentacled bud axis that also included a parental tissue wedge, however, produced distal budding in 22 of 31 grafts.

To determine if parental tissue alone, rather than parental *and* bud tissue, could direct bud separation from the distal surface of a host amputated subhypostomally, we used portions of parental body column as donor tissue. Parental budding region directed distal budding (9 of 30 cases), while parental gastric region did not (0 of 31 cases).

Influence of parental hypostome on bud development and separation

According to the gradient theory (Weimer, 1928), the dominant parental hypostome and adjacent tissue normally prevent budding from regions more distal than the budding region. Browne (1909) and Li and Yao (1945) found that bud donor tissue grafted to the gastric region of a parental host initiated an axial outgrowth but did not cause the development of a bud that separated. However, Clarkson and Wolpert (1967), grafted an early bud hypostome rudiment to the parental gastric region and produced bud development with separation. Based on these observations and the previous results (where a graft of bud protrusion with attached parental body wedge led to distal budding) we tested whether a bud protrusion with an attached parental wedge could direct bud detachment when grafted laterally in the distal-most gastric region of a parental host (*i.e.* very near the dominant parental hypostome).

In 22 of 32 grafts, the bud completed its development and detached, showing

that bud protrusions with parental axial tissue can lead to bud development and separation from a parental host in a region more distal than the budding region, and more closely associated with the parental hypostome. Coincident with bud separation, the parental organism formed a slight lateral protrusion at the base of the developing bud. This remained for the rest of the experiment.

A parental hypostome grafted alone did not lead to formation of a bud that separated from the parental body column, nor did it direct distal budding from a parental animal. To determine if a parental hypostome would prevent separation of a bud which had begun development, a parental hypostome was grafted to a zero-tentacled bud axis that had been amputated proximal to the presumptive hypostomal region. In none of 30 grafts did a parental hypostome interfere with completion of bud development and separation.

Cellular distributions

The grafting experiments indicated that tissue from the budding region of the parental body column was necessary to direct bud detachment. To examine a possible cellular basis for this ability, we macerated tissues from several body regions and identified and categorized the disaggregated cells from each sample. Samples of parental budding region annuli and bud protrusions with parental body column were compared with parental hypostomes (without tentacles), zero-tentacled bud hypostomes, two-tentacled bud hypostomes with tentacles, four-tentacled bud hypostomes with tentacles, one-third annuli of gastric regions, and peduncle annuli.

Table II gives the number of cells of various types in selected body regions, expressed as a proportion of the number of epithelial cells. The percentage of epithelial cells in a bud remains fairly constant throughout its development (Bode *et al.*, 1973). The cell types counted were epithelial cells (epitheliomuscular cells

TABLE II

Cell-type proportions from selected hydra body regions. The epithelial-cell proportion column gives the epitheliomuscular and digestive cell count/total cell count, with raw data in parentheses. In all other columns the proportion is the proportion of the cell type listed to epithelial cells.

Body region	Epithelial cell proportion	Little I-cells	Big I-cells	Nerve cells	Nematoblasts, nematocytes, and nerve cells	Secretory cells
Parental hypostome	0.45 (367/839)	0.16	0.27	0.34	0.56	0.24
Four-tentacled bud hypostome	0.40 (333/841)	0.35	0.41	0.29	0.66	0.11
Two-tentacled bud hypostome	0.50 (400/793)	0.14	0.41	0.14	0.36	0.07
Peduncle	0.83 (604/728)	0	0	0.05	0.12	0.09
⅓ gastric region annulus	0.26 (231/876)	1.13	0.78	0.06	0.76	0.12
⅓ budding region annulus	0.34 (277/807)	0.81	0.65	0.02	0.43	0.03
Bud protrusion with parental body column	0.40 (312/782)	0.42	0.62	0.07	0.44	0.03
Zero-tentacled bud hypostome	0.31 (254/826)	0.59	1.09	0.05	0.49	0.08

and digestive cells), nerve cells, "big" interstitial (I) cells (which encompass the stem cell population and cells which may differentiate into nerves), "little" I-cells (which may differentiate into nematoblasts and ultimately nematocytes), nematoblast cells, nematocytes, and secretory cells (David, 1973).

The proportions of big I/epithelial cells, nerve/epithelial cells, and I-cell derivatives (nematoblasts, nematocytes, and nerves)/epithelial cells were similar in the budding region and bud protrusion. The big I-cell/epithelial proportion always was considerably <1 (0.65 and 0.62 respectively); the nerve cell/epithelial proportions were very low (0.02 and 0.07 respectively), and the I-cell derivatives/epithelial proportions were slightly <0.50 (0.43 and 0.44 respectively). Based on morphological or temporal similarities to the budding region or bud protrusion, gastric region tissue and zero-tentacled bud hypostome also might have been expected to direct distal budding. Each of these regions shared two of the three cellular patterns characterizing the budding region and bud protrusion. The gastric region showed a higher proportion of I-cell derivatives (0.76) than the noted <0.50 level. The zero-tentacled bud showed a high big I-cell proportion (1.09) in its presumptive hypostomal area. Older hypostomal tissue samples contained higher proportions of nerve cells and lower proportions of big I-cells than the samples involved in bud separation.

Discussion

A hydra bud is distinguished from a secondary axis of a colonial coelenterate by its formation of a peduncle and a basal disc, and by its subsequent individuation. From this investigation we conclude that the ability to direct bud separation from a parental axis resides within the parental body column. While various isolated tissues of *H. oligactis*, including isolated bud tissue, can form peduncles and basal discs, bud tissue apparently does not direct the natural separation of a secondary axis from a parental body column.

Hypostomal tissues from bud-protrusion through parental stages were grafted to the distal surface of parental hosts, to test rigorously whether these tissues can direct bud separation. Neither homografts nor heterografts of parental hypostomes resulted in distal budding (Sersig, unpublished). This observation served as a control and in particular indicated that the surgical manipulations alone would not lead to distal budding. Bud hypostomes also did not direct distal budding. However, bud protrusions grafted with small amounts of adjacent parental body column, and subhypostomally amputated two-tentacled bud axis with adjacent parental body column, did lead to bud separation.

These results suggest a requirement for parental body column tissue in directing bud separation. This hypothesis was verified by the observation that parent budding region annuli, but not gastric region annuli, lead to distal budding. Thus, parental body tissue is both required and sufficient for bud separation.

Numerous additional experiments challenged isolated parental and bud tissue to form peduncle and basal disc, structures necessary to complete bud individuation. These data collectively further substantiate an involvement of parental body column in directing bud separation.

A cellular basis for the capacity to direct bud separation also was sought, by comparing regions capable of directing distal budding (budding region and bud protrusion) to regions not implicated in bud separation. A combination of three cellular distributions was unique to the budding region and bud protrusion: considerably fewer big I-cells than epithelial cells, few nerve cells, and an I-cell de-

rivative/epithelial cell proportion slightly <0.50. To assess the significance of these data, each distribution must be evaluated individually and comparisons made between the cell compositions presented here and those in homologous tissue of non-budding hydra mutants (Moore and Campbell, 1973).

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